

Applicant: Hiroaki Shizuya  
Application No.: 09/896,509  
Filed: June 28, 2001  
Page 7

### REMARKS

Claims 1-55 were pending prior to this Response, the restriction requirement mailed September 17, 2002 having been withdrawn. By the present communication, the Abstract on page 32 has been amended to provide a definition for the abbreviation "BAC". In addition, claims 1, 2, 25, 43, 46, 47 and 55 have been amended to define Applicant's invention with greater particularity as shown in Exhibit A, with no claims being added or cancelled. No new matter is added, the new claim language being fully supported by the Specification and original claims. Accordingly, claims 1-55 are currently pending.

#### **The Rejection Under 35 U.S.C. § 112, Second Paragraph**

Applicant respectfully traverses the rejection of claims 2, and 25-55 under 35 U.S.C. § 112, Second Paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. The Examiner asserts that the phrase "a corresponding essential gene" as used in claims 25 and 55 is indefinite due to alleged lack of clarity to what "corresponding" refers. However claims 25 and 55 have been amended to delete the term "corresponding" thereby rendering the rejection moot as to claims 25 and 55.

The Examiner asserts that recitation in claims 46-48 regarding "introduction of BAC into the host cell" is allegedly unclear due to lack of sufficient antecedent basis because the BAC is introduced into the test cell according to the claims. To overcome this inadvertent error identified by the Examiner, Applicant has amended claims 46-48 to clarify that the BAC is inserted into the test cell, thus rendering moot the grounds for the rejection.

Applicant: Hiroaki Shizuya  
Application No.: 09/896,509  
Filed: June 28, 2001  
Page 8

In addition, the Examiner asserts that claims 2, 25 and 43 (and claims 26-42 and 44-55 dependent thereon) are indefinite as allegedly omitting an essential element, namely, "how the gene is obtained by homology with the identified essential chromosomal gene in the test cell" (Office Action, page 3). However, claims 2, 25 and 43 have been amended to clarify that the "essential chromosomal gene in the test cell" is obtained by performing a sequence comparison of the DNA in the dead test cell and the known genome of the host cell to locate a gene in the known segment of DNA of the haploid test organism (e.g., a pathogenic bacterium) that has been disrupted by the transposon. An essential premise of the invention methods is that essential genes are highly conserved among bacterial species and the segment of DNA in the BAC is known (e.g., for each different BAC). Therefore, sequence comparison of the known genome of the host cell with that of a dead or replication impaired test cell can be used to identify a disrupted (i.e. mutagenized) gene as being "an essential gene.". Thus, the identified disrupted gene will be an essential gene, e.g., a gene necessary to replication and/or growth of the test organism.

#### **The Objection Under 35 U.S.C. § 112, First Paragraph**

Applicant respectfully traverses the rejection of claims 1-12, 14-34, 36-45, 47 and 49-55 as allegedly lacking enablement for use of "any host cell" (Office Action, page 3). The Examiner states that the description of the invention is enabling only for host cells having the following characteristics: 1) the DNA sequence of the entire genome must be known; 2) the host cell must be haploid while replicating and resistant to environmental conditions to which it will be subjected to prevent BAC replication; and 3) the host cell must stably propagate the transformed

Applicant: Hiroaki Shizuya  
Application No.: 09/896,509  
Filed: June 28, 2001  
Page 9

DNA. Therefore, the Examiner asserts that the invention is unpredictable unless the host is characterized according to these characteristics.

However, Applicants claims are not broadly drawn to "any host cell." All pending claims require that the host cell is a haploid host cell whose genome is known (i.e., mapped so that the location of genes in the host genome is known), which is capable of being transformed by artificial means, and is capable of undergoing DNA recombination. In addition, with regard to the Examiner's concern regarding resistance of the host cell to environmental conditions to which BAC is sensitive, all claims presently recite "wherein replication of the BAC in the test cell is sensitive to an environmental condition that selectively prevents replication of the BAC in the host cell." Applicant respectfully submits that inherent in the phrase "that selectively prevents replication of the BAC in the host cell" is the premise that the "environmental condition" does not prevent replication of the host cell, otherwise the term "selectively" in claims 1, 25 and 43 would have to be ignored.

Accordingly, Applicants respectfully submit that, although *E.coli* is the preferred host cell for use in the invention methods, Applicant teaches that additional host cells whose genome is known and which can be used in practice of the invention are *Salmonellae* and *B. subtilis*. The burden is on the Examiner to provide evidence to support the Examiner's conclusion that expression of BAC in *Salmonellae* and *B. subtilis* is unpredictable and would therefore involve undue experimentation. This burden has not been met in the present Office Action. Accordingly, Applicant respectfully submits that to restrict the scope of the claims to the Examples would unfairly limit Applicant's invention and reconsideration and withdrawal of the rejection for lack of enablement under 35 U.S.C. § 112, First Paragraph, is respectfully requested.

Applicant: Hiroaki Shizuya  
Application No.: 09/896,509  
Filed: June 28, 2001  
Page 10

In addition, Applicant respectfully traverses the rejection of claims 1-12, 14-34, 36-45, 47 and 49-55 under 35 U.S.C. § 112, First Paragraph, for allegedly lacking sufficient description so as to convey to those of skill in the art that the inventor had possession of the invention at the time the application was filed. The Examiner acknowledges that the description requirement for genus claims may be satisfied through sufficient description of a representative number of species as well as by reduction to drawings, disclosure of relevant identifying characteristics, functional characteristics coupled with known or disclosed correlations between function and structure, or by a combination of such characteristics (Office Action, page 6). Applicant respectfully submits that in the present application a number of relevant identifying characteristics and correlations between structure and function that apply to the host cell used in the invention methods are clearly described by words and by drawings (i.e., Figures 1 through 4).

For example, Applicant teaches and the claims require that suitable host cells are limited to those whose mode of replication is haploid and the nexus between this structural requirement and the operation of the invention, especially the creation of merodiploid cells, is made abundantly clear throughout the application and the Figures. In addition, Applicant also teaches that the genome of the haploid host cell should be known (e.g., to allow sequence comparison of the DNA of the host cell with a DNA segment obtained from a test cell identified as having a lethal mutation in an essential gene). Applicant disagrees with the Examiner's assertion that "the identity of any host cell whose genome sequence is known must be empirically determined" (Office Action, page 6). Those of skill in the art could readily determine, without sequencing the genome of a haploid cell, whether its genomic sequence has already been mapped from the scientific literature. There is no "uncertainty" to those of skill in the art regarding such a readily ascertainable scientific fact. In addition, Applicant does not have any obligation to describe any but those haploid host cells whose genome was known at the filing date of the application.

Applicant: Hiroaki Shizuya  
Application No.: 09/896,509  
Filed: June 28, 2001  
Page 11

Therefore, Applicant respectfully submits that the Specification provides sufficient "relevant identifying characteristics" for those of skill in the art to understand that the Applicant had possession of the invention at the filing date of the application. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, First Paragraph of claims 1-12, 14-34, 36-45, 47 and 49-55 for lack of description of the claimed invention in the Specification are respectfully requested.

#### **The Objection to Claims 13 and 35**

Applicant respectfully traverses the objection to claims 13 and 35 as dependent upon a rejected base claim. In view of the above amendments and remarks, Applicant respectfully submits that all objections and rejections have been overcome and that claims 1 and 25 from which claims 13 and 35 ultimately depend are now allowable.

In view of the above amendments and remarks, Applicant respectfully requests withdrawal of all rejections and objections to the claims and Specification. If the Examiner

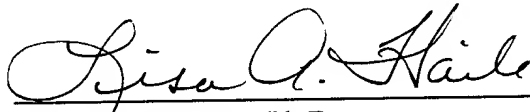
PATENT  
ATTORNEY DOCKET NO.: CIT1390-1

Applicant: Hiroaki Shizuya  
Application No.: 09/896,509  
Filed: June 28, 2001  
Page 12

would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

Date: March 7, 2003



Lisa A. Haile, J.D., Ph.D.  
Registration No.: 38,347  
Telephone: (858) 677-1456  
Facsimile: (858) 677-1465

**USPTO CUSTOMER NUMBER 28213**  
**GRAY CARY WARE & FREIDENRICH LLP**  
4365 Executive Drive, Suite 1100  
San Diego, California 92121-2133

Enclosure: Exhibit A



PATENT  
ATTORNEY DOCKET NO.: CIT1390-1

Applicant: Hiroaki Shizuya  
Application No.: 09/896,509  
Filed: June 28, 2001  
Exhibit A: Page 1

## EXHIBIT A

### Version with Markings to Show Changes Made

#### In the Specification

Please amend the Abstract on page 32 to read as follows:

Methods are provided for the rapid identification of essential or conditionally essential DNA segments in any species of haploid cell (one copy chromosome per cell) that is capable of being transformed by artificial means and is capable of undergoing DNA recombination. The bacterial artificial chromosome (BAC) system is used to provide to the prokaryotic host cell an additional copy of a known segment of DNA of the host cell (or of another prokaryotic cell whose genome is known) to construct merodiploid test cells wherein the chromosomal region of the host cell that is homologous to the DNA contained in the BAC becomes diploid. Alternatively, due to the high homology in essential genes of prokaryotes, the DNA contained in the BAC can be derived from a prokaryote other than the host cell. A transposon is then delivered randomly to the merodiploid cell. Due to presence of the BAC carrying a segment of homologous DNA, the merodiploid cell will survive replication if the transposon disrupted gene on the host chromosome is replaced by a second normal gene existing on the particular BAC contained within the particular host cell. However, in the event the BAC carrying the second normal gene copy is lost during replication or the BAC replaces a normal gene in the host cell with a defective copy due to recombination, inhibition of growth or lethality of the test cell will result. This system offers an enhanced means of identifying essential function genes in diploid pathogens, such as gram-negative and gram-positive bacteria.

Applicant: Hiroaki Shizuya  
Application No.: 09/896,509  
Filed: June 28, 2001  
Exhibit A: Page 2

**In the Claims**

Please amend the claims 1, 2, 25, 43, 46, 47 and 55 as follows:

1. (Amended) A method for identifying an essential chromosomal gene in a haploid test organism, said method comprising:

constructing a BAC-carrying merodiploid test cell by transforming a wild-type haploid host cell whose genome is known, which is capable of being transformed by artificial means and undergoing DNA recombination, with a bacterial artificial chromosome (BAC) carrying a known segment of DNA of the haploid test organism, which segment is homologous to a known segment of chromosomal DNA in the host cell, and wherein replication of the BAC in the test cell is sensitive to an environmental condition that selectively prevents replication of the BAC in the host cell;

inserting randomly a bacterial transposon into the merodiploid test cell so as to disrupt function of a gene therein;

culturing one or more of the BAC-carrying merodiploid test cells in a suitable culture medium while introducing the environmental condition so as to transform the merodiploid test cells into haploid test cells; and

identifying one or more of the haploid test cells that contain transposon-mutagenized DNA in an essential chromosomal gene therein.

2. (Amended) The method of claim 1 further comprising obtaining the essential chromosomal gene in the test cell by [homology with] identifying by sequence comparison with the known genome of the host cell a gene in the known segment of DNA that has been disrupted by the transposon.

Applicant: Hiroaki Shizuya  
Application No.: 09/896,509  
Filed: June 28, 2001  
Exhibit A: Page 3

25. (Amended) A method for screening bacterial genes in a pathogenic bacterium whose genome is known to select compounds with putative antibiotic activity, said method comprising:
- constructing a BAC-carrying merodiploid test cell by transforming a wild-type haploid host cell whose genome is known, which is capable of being transformed by artificial means and capable of undergoing DNA recombination, with a BAC that carries a known segment of DNA of a pathogenic bacterium[, which segment is homologous to a segment of chromosomal DNA in the host cell], and wherein the BAC in the test cell is sensitive to an environmental condition that selectively prevents replication of the BAC in the test cell;
  - inserting randomly a transposon into the merodiploid test cell so as to disrupt function of a gene therein;
  - culturing one or more of the merodiploid test cells in a suitable culture medium while introducing the environmental condition;
  - identifying one or more test cells that do not survive subjection to the environmental condition as containing the transposon in an essential chromosomal gene therein;
  - obtaining [a corresponding] the essential gene in the known segment of DNA of the pathogenic bacterium by [homology with the identified essential chromosomal gene in the test cell] identifying by sequence comparison with the known DNA of the host cell a gene in the known segment of DNA of the pathogenic organism has been disrupted by the transposon; and
  - screening the [corresponding] essential gene obtained from the pathogenic bacterium or a bacterial protein encoded by the corresponding essential gene against putative antibiotic compounds to determine those compounds that bind to or interrupt function of the [corresponding] essential gene or the bacterial protein, wherein such a compound is a candidate antibiotic against the pathogenic bacterium.

Applicant: Hiroaki Shizuya  
Application No.: 09/896,509  
Filed: June 28, 2001  
Exhibit A: Page 4

43. (Amended) A method for identifying an essential chromosomal gene in a haploid test organism, said method comprising:

constructing a BAC carrying a known segment of DNA of the haploid test organism, which segment is homologous to a known segment of chromosomal DNA in a haploid host cell whose genome is known, which is capable of being transformed by artificial means and undergoing DNA recombination;

inserting randomly a bacterial transposon into the BAC so as to disrupt function of a gene in the segment of chromosomal DNA;

introducing the BAC into the a haploid [test] host cell to create a merodiploid test cell;

culturing the merodiploid test cell in a suitable culture medium such that the BAC in the test cell is sensitive to an environmental condition that selectively prevents replication of the BAC in the test cell;

identifying one or more BAC-carrying merodiploid test cells that do not survive in culture as containing the transposon in an essential chromosomal gene therein; and

obtaining the identity of the essential chromosomal gene by [homology with] identifying a gene that has been disrupted by the transposon by sequence comparison between the known genome of the host cell and the known segment of DNA inserted into the BAC.

46. (Amended) The method of claim 43, wherein the transposon is inserted randomly into the BAC *in vitro* prior to introduction of the BAC into the [host] test cell.

47. (Amended) The method of claim 46, wherein the known segment of DNA is linearized prior to introduction into the [host] test cell.

Applicant: Hiroaki Shizuya  
Application No.: 09/896,509  
Filed: June 28, 2001  
Exhibit A: Page 5

55. (Amended) The method of claim 54, wherein the method further comprises screening a [corresponding] essential gene obtained from the pathogenic bacterium or a bacterial protein encoded thereby against putative antibiotic compounds to determine those compounds that bind to or interrupt function of the corresponding essential gene or the bacterial protein, wherein such a compound is a candidate antibiotic against the pathogenic bacterium.